BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348-0521, ISSN (E): 2454-4728
Vol. 5, Issue 12, Dec 2017, 59-64

CLARIFICATION OF CARAMBOLA FRUIT JUICE USING A COMMERCIAL ENZYME - OPTIMIZATION OF CONDITIONS

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ABSTRACT

In the Central Composite Design, the method employed was Response Surface Methodology, which is used in the analysis for optimizing the conditions of enzymatic treatment, on physical characteristics. Pectinase was used to treat carambola juice at various enzyme concentration i.e., 0.01 – 0.1%, Incubation time of 30 to 120 mins and incubation temperature of 30 to 50°C. Central Composite Design of second order was employed that had treatment conditions as independent variables, which has a major impact on physical characters such as turbidity, viscosity and filterability. With coefficient of determination R^2, the significant regression models are describing the changes on the physical characters, with respect to the independent variables that were established. According to the response surface methodology and the plots, the optimum treatment conditions for clarification of carambola juice were incubation temperature of 40°C, incubation time of 75 mints and enzyme concentration of 0.055%.

KEYWORDS: Enzymatic Treatment, Turbidity, Viscosity & Filterability

INTRODUCTION

In the developed and developing countries, juices like grape, mango, lime and blends are traditionally established well. These fruits are used to have value added produce and are widely produced to extend its marketability, mainly in exports. Recently, there is a great attention on juices and products of tropical fruits. The largest exporters of carambola or star fruit in the world are Malaysia, since 1989 and they have been exporting to countries such as Europe, where this commodity is a major product.

In the east, one of the popular tropical fruit is Carambola, which is relatively expensive. These are mostly consumed as fresh or served as fresh juices, or in blends as flavoured ingredients. This is juicy and delicious with an exclusive flavour and an attractive flesh, sweet and is slightly acidic. It is from the family Oxalidacceace, scientifically known as *Averrhoa carambola L*. Five colour indices were used to indicate the various stages of maturity. This is highly rich in Vitamin A and C, amounting to more than 25 mg per 100 mg of the fresh fruit. This varies from light sour to sweet in flavour.

Products or value added products such as fruit juices and blends have polysaccharides which are colloids. The viscosity and turbidity of the juice is caused by these polysaccharides like pectin and starch. The major problem that is faced in the preparation of juices is cloudiness, which is due to the presence of pectin; Enzymaticdepectinization can be used for removal. Due to the pectin which has fiber like molecular structure, the clarification process is difficult as it is associated with plant polymers and cell debris. The flocculation of pectin protein complex is caused by hydrolysation of pectin, by pectinase in enzymatic depectinization. On analysis, 0.15% wt of pectin was present in the carambola juice. From the pectinase, the treated relating juice determines the lower amount of pectin and the lower viscosity. For the hydrolysis of pectin by enzymatic treatment, it is influenced by numerous factors like incubation time, enzyme concentration and incubation temperature. Central Composite Design (CCD) is the most suitable approach, for determining optimum conditions of the processes. In the design of experts, response surface methodology is the most efficient and effective optimization process. To consecutively solve and determine the multivariate equations from appropriate experimental design, this is used as a suitable tool for the expenditure of quantitative data. In tropical fruit juice production, this method is very widely used for the optimization of process.

MATERIALS AND METHODS

Materials

Fresh Carambola or starfruit, for the enzymatic treatment of carambola juice and Pectinase Ultra SP-L.

Methodology

Methodology to be followed in the Design Expert Software: Choose the Central composite design in the Response surface methodology. Then, set parameters are the independent variables, which are enzyme concentration, incubation time and incubation temperature that are to be determined, then set the response activity such as filterability, turbidity and viscosity. Then, apply it in the Design Expert software Version 10.

Experimental Design

To study the combined effect of three independent variables that is enzyme concentration, incubation temperature and incubation time the experiment was employed with quadratic model based upon the central composite deign. These independent variables are coded as A, B and C. These variables are responsible for the mechanism of the enzyme activity. According to the central composite design a number of 25 combinations having five replicates were carried out for these chosen variables in table 1. The dependent variables (y) measured were filterability (y1), turbidity (y2) and viscosity (y3) of the carambola juice. The dependent variables are expressed individually as the function of independent variables known as response function. Using the second order polynomial function the variance for each factor was assessed and partitioned into linear, quadratic and interactive components and are presented as follows.

$$Y1 = 0.081186 – 0.092185A – (3.77368E +004)B – (9.74588E + 005)C + (3.3333E – 003)AB – (1.60494E + 003)AC + (4.44444E – 006)BC + 0.29890A^2 – (3.07018E + 007)B^2 – (3.03227E – 008)C^2$$

$$Y2 = 0.018233 – 0.21403A + (7.62125E - 004)B + (2.16776E - 004)C - (7.222222E + 003)AB + (2.34568E - 003)AC - (5.000000E + 006)BC - 0.29938A^2 – (6.23782E + 007)B^2 – (4.63986E – 008)C^2$$

$$Y3 = =8.87231 – 21.99514A – 0.46626B + 0.59204C + 0.70278AB – 0.2796AC – 0.012964BC + 128.27473A^2 + 0.016892B^2 + (1.28275E – 004)C^2$$

Table 1: Experimental Design Matrix Developed by Response Surface Methodology

Std	Group	Run	Factor 1 A:Enzyme c... %	Factor 2 b:Incubation ... degree C	Factor 3 C:Incubation ... mints	Response 1 Filterability s^{-1}	Response 2 Turbidity NTU	Response 3 Viscosity Pa s
10	1	1	0.055	40	75	0.062	0.019	12.4
9	1	2	0.055	40	75	0.072	0.007	2.32
11	1	3	0.055	40	75	0.069	0.02	5.2
23	2	4	0.055	40	75	0.071	0.009	3.69
24	2	5	0.055	40	75	0.064	0.015	5.5
25	2	6	0.055	40	75	0.073	0.009	3.67
5	3	7	0.01	50	30	0.076	0.01	2.47
7	3	8	0.01	50	120	0.075	0.016	3.5
6	3	9	0.1	50	30	0.073	0.011	3.5
8	3	10	0.1	50	120	0.072	0.02	3.5
22	4	11	0.055	40	75	0.075	0.012	3
21	4	12	0.055	40	75	0.055	0.025	9
20	4	13	0.055	40	75	0.071	0.015	3
4	5	14	0.1	30	120	0.048	0.042	25.9
1	5	15	0.01	30	30	0.066	0.01	2.8
3	5	16	0.01	30	120	0.07	0.009	28.4
2	5	17	0.1	30	30	0.07	0.008	3.8
19	6	18	0.055	40	152.942	0.071	0.008	3.8
16	6	19	-0.0229423	40	75	0.071	0.007	3.8
18	6	20	0.055	40	-2.94229	0.064	0.008	3.8
17	6	21	0.132942	40	75	0.068	0.011	3.8
13	7	22	0.055	22.6795	75	0.069	0.013	3.54
12	7	23	0.055	22.6795	75	0.072	0.012	3.89
15	8	24	0.055	57.3205	75	0.063	0.014	25.7
14	8	25	0.055	57.3205	75	0.067	0.011	3.8

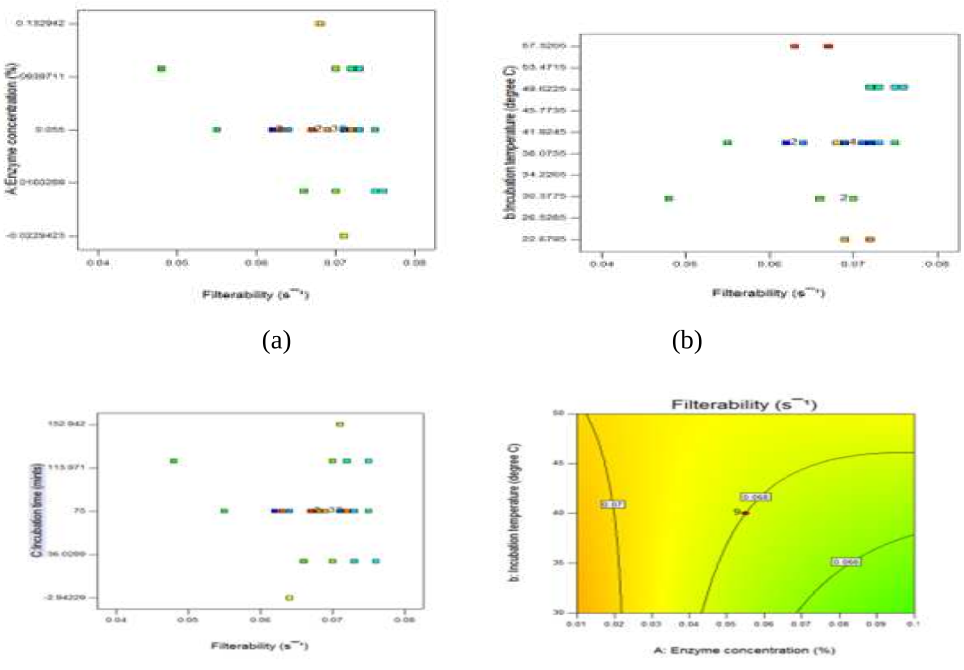
RESULTS AND DISCUSSIONS

Statistical Analysis

For the three response variables that is filterability, turbidity and viscosity the analysis of variance and experimental values are obtained under different treatment conditions. It characterised that it was adequate for all response variables which are developed in the response surface model. The reaction was explained well by the regression model for all these response variables as R^2 was higher.

Filterability

Filterability was related to linear effect of the enzyme concertation, incubation temperature and time. Filtration has an interaction effect among incubation time and enzyme concentration. In the juice, the filterability was found to increase with an increase in enzyme concentration having incubation time at its lowest.



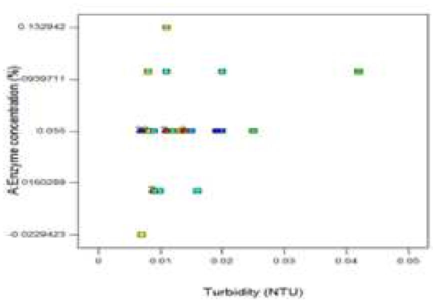
(a) (b)

(c) (d)

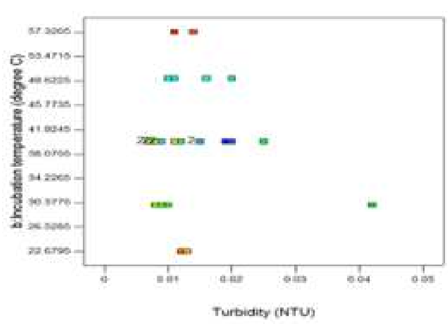
Figure 1: (a), (b) and (c) Shows the Relationship of Filterability with that of the Physical Characteristics and (d) Shows the Relationship of Filterability with that of Incubation Temperature and Enzyme Concentration

Turbidity

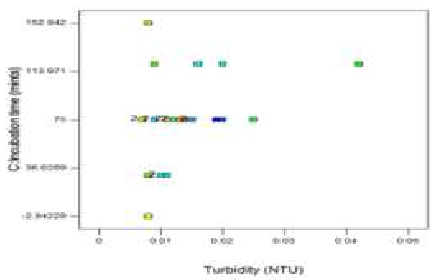
Turbidity was majorly a function of the enzyme concentration. An increase in enzyme concentration drastically decreases turbidity. The turbidity decrease evidently as enzyme concentration was raised, irrespective of incubation temperature and time of the enzyme treatment. The interaction of enzyme concentration and incubation time was also important. The interaction effect was positive on turbidity.



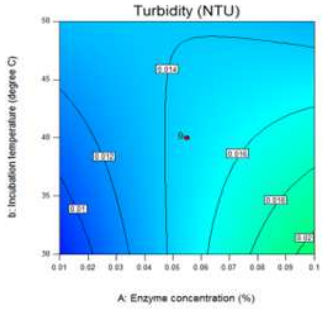
(a)



(b)



(c)

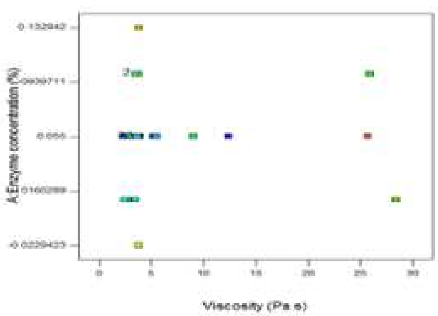


(d)

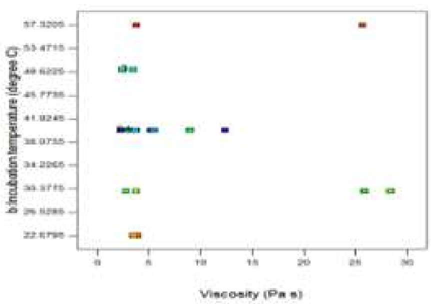
Figure 1: (A), (B) and (C) Shows the Relationship of Turbidity with that of the Physical Characteristics and (D) Shows the Relationship of Turbidity with that of Incubation Temperature and Enzyme Concentration

Viscosity

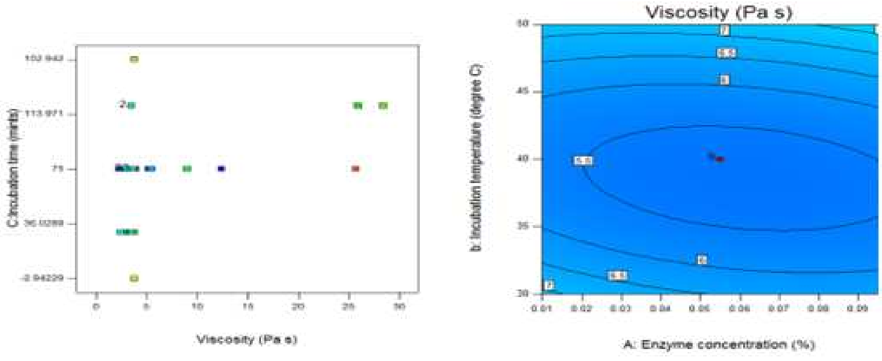
Viscosity was reduced considerably due to higher enzyme concentration. Enzyme concentration and incubation time has a significant interaction effect with viscosity.



(a)



(b)



(c) (d)

Figure 1: (a), (b) and (c) shows the Relationship of Viscosity with that of the Physical Characteristics and (d) shows the Relationship of Viscosity with that of Incubation Temperature and Enzyme Concentration

Optimization

The optimum conditions for the process of clarification which facilitates the preceding filtration is to yield minimum viscosity, minimum turbidity and maximum filterability. To obtain minimum levels of viscosity and turbidity, and maximum levels of filterability, there are numerous combinations available, having different variables at different conditions. It was done to obtain all the contour plots of the superimposition, as each dependant variable did not accurately fall in the same region in the optimum response. The best combination of the response functions for the process variables are incubation temperature at 40ºC, incubation time for 75 mints and enzyme concentration of about 0.055%. The final polynomial is used to determine the response functions which were turbidity, filterability and viscosity.

CONCLUSIONS

These different conditions like enzyme concentration, incubation temperature and incubation time for the enzyme treatment revealed that, these variables affect the filterability, turbidity and viscosity of the carambola juice. The enzyme treatment conditions can be related by second order polynomials. In order to obtain desirable conditions for the properties of the carambola juice, that will be suitable for the membrane based clarification, the optimum operating values have been determined graphically.

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